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Efficacy of PCR and Other Diagnostic Methods for the Detection of Respiratory Adenoviral Infections

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Five methods were evaluated for the detection of adenovirus directly from nasopharyngeal aspirates (NPA), including conventional and rapid virus culture, two antigen detection tests, and the polymerase chain reaction (PCR). NPA specimens were obtained from 269 military conscripts suffering from an acute respiratory infection during an adenovirus outbreak. In 133 cases, paired blood specimens were also available. Virus culture followed by a hexon-specific immunofluorescence revealed 159 (59%) adenovirus-positive specimens and it was used as a reference method. In comparison to conventional culture, a rapid 2-day culture method had a sensitivity of 71%. The sensitivities of an enzyme immunoassay and time-resolved fluoroimmunoassay were 53% and 46%, respectively. The PCR method employing Ad7 hexon-specific primers showed a high sensitivity of 94%, and revealed an additional 15 (6%) specimens that could not be confirmed by virus culture. Serology based on significant adenovirus antibody rises had a diagnostic efficacy nearly equal to the virus culture and PCR methods, but a relatively high number of discordant results was found. The present study demonstrates that PCR is a very sensitive rapid diagnostic method for detecting adenovirus specific DNA in NPA specimens of adults. ***J. Med. Virol. 59:66–72, 1999.*** © 1999 Wiley-Liss, Inc.

KEY WORDS: virus culture; antigen detection; DNA detection; virus serology

INTRODUCTION

Adenoviral infections are common in all age groups of the general population. In military settings upper respiratory tract infections caused by adenoviruses may occur sporadically or as epidemics, which can last for several weeks [Horwitz, 1996]. Sporadic adenoviral cases, in particular, may be indistinguishable from infections caused by other respiratory pathogens such as influenza, parainfluenza, and respiratory syncytial viruses, as well as by certain bacteria. Mixed infections caused by two or more different pathogens are encountered frequently [Burman et al., 1991]. In any case, the knowledge of the presence or absence of adenovirus is useful when the decision on starting antimicrobial therapy is made. Also, the correct etiological diagnosis is important for epidemiological purposes.

Currently 49 different adenovirus serotypes, which have been classified into subgroups A, B, C, D, E, or F, have been identified. However, most human adenoviral infections are associated with only one-third of these serotypes [Horwitz, 1996]. Depending on the serotype, there are considerable differences in the tissue specificity and virulence of adenoviruses. The different outcomes of adenovirus infections are not very well understood: for example, why the infection is self-limiting in some individuals and becomes persistent in others [Flomenberg et al., 1997; Mentel et al., 1997]. In countries where vaccination against adenoviruses is not used, acute respiratory disease (ARD) due to adenoviruses is a significant cause of morbidity, especially in the army [Mäntyjärvi, 1966; Dudding et al., 1973]. The etiological agents of ARD in outbreaks among military servicemen have often been of adenoviruses type 7 (Ad7) or type 4 (Ad4) [Horwitz, 1996].

The material used in this study was obtained from 269 military conscripts with an acute respiratory infection. The rate of adenovirus infections in these patients was very high, enabling a reliable comparison of several adenovirus-specific diagnostic methods, including conventional and rapid virus cultivation methods, detection of adenovirus-specific proteins by an enzyme immunoassay (EIA) and by a time-resolved fluoroimmunoassay (TR-FIA), and an adenovirus hexon gene-specific PCR assay.

MATERIALS AND METHODS

Patients and Specimens

A total of 269 nasopharyngeal aspirates were collected from military conscripts suffering from an acute upper or lower respiratory tract infection during 4

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months from the beginning of January to the end of April 1991. NPA specimens were collected by suction through the nostrils using a mucus extractor (UnoPlastA/S, Hundestadt, Denmark). The specimens in the carefully closed containers without transport medium arrived at the laboratory with an average delay of 1 day. Two hundred twenty-three NPA specimens were received from the Central Military Hospital in Helsinki and 46 specimens from different garrisons located mainly in the southern parts of Finland. Paired serum specimens were obtained from 133 of the 269 patients. Acute-phase sera were collected at an average of 3.8 days after the onset of symptoms and convalescent phase sera 14 days later. The criteria for inclusion of the patients in the study were: the patients had fever and respiratory symptoms that had lasted less than 6 days. However, 14 NPA samples were taken at a later phase (= 7 days after onset) of the infection. These specimens were also analyzed and they gave us an opportunity to estimate the efficiency of various diagnostic methods in relation to the sampling time.

The NPA samples were homogenized with dithiotreitol solution (final concentration 5 mM) as described previously [Kleemola et al., 1993]. From each homogenized NPA specimen 0.5 ml was first reserved for the PCR assay. For TR-FIA and EIA, the NPA specimens were further diluted 1:2.5 in phosphate-buffered saline (PBS, pH 7.4) containing 20% fetal calf serum, 2% Tween 20, and 0.1% sodium azide. The diluted samples in vials were dispersed by ultrasound (300 W, 2 min) using a sonicator with a horn cup filled with water (VibraCell, Sonics and Materials, CT).

Virus Culture

A human lung A549 carcinoma cell line (ATCC CCL 185) was used for the culture of adenovirus from the NPA samples (0.1 ml of each sample in duplicates). During virus propagation the tube cultures were maintained in 1 ml of Eagle's Minimum Essential Medium (MEM) supplemented with 2% inactivated fetal calf serum, antibiotics, and 20-mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid; pH 7.4). The cell cultures were inspected daily for the development of a cytopathic effect (CPE) up to 2 weeks. When full CPE was detected (range, 2 to 14 days), detached cells were collected by pelleting. The cells remaining in the tube culture were collected into 0.1 ml of a detachment buffer (10-mM Tris-HCl, pH 7.4, 5-mM EDTA, and 10-mM KCl). The cells were pooled and the cell suspensions were stored at –70°C until a confirmatory culture with immunostaining was performed.

A confirmatory culture for adenoviruses was carried out in A549 cells grown on glass coverslips in 24-well plates. The confluent cell monolayers on coverslips were inoculated with 1:40 and 1:120 dilutions of cell extracts of primary A-549 cultures. After a 2-day incubation, the cells were fixed and immunostained as described previously [Waris et al., 1990]. Briefly, the cells were washed with phosphate-buffered saline (PBS), fixed for 15 min with 4% paraformaldehyde (Riedel-de Haen AG, Seelze-Hannover, Germany), made permeable for 5 min with 0.1% Triton X-100, washed and treated with 0.5% bovine serum albumin (BSA, Sigma, St. Louis, MO) in PBS for at least 30 min. Cells were stained with rabbit anti-hexon antisera for 30 min, washed and stained with FITC-conjugated anti-rabbit IgG $F(ab')_2$ fragment (Boehringer Mannheim, Germany). An Axiophot photomicroscope was used to detect hexon-specific immunofluorescence. Twenty-five randomly selected adenovirus culture-positive specimens received from different garrisons were subjected to adenovirus typing using a neutralization test [Kasel, 1992].

Rapid Virus Culture Method

NPA samples were directly cultivated on glass coverslips in multiwell plates (Greiner Laborteknik) using a technique essentially the same as that described above for serological confirmation of adenovirus culture-positive specimens except that the lowest dilution of the NPA sample was 1:20.

Adenovirus DNA Amplification

Polymerase chain reaction for the detection of adenovirus was performed with Ad7-specific primers, which were selected from the same region of the hexon sequence, as were Ad2-specific primers H1 and H2 used by Allard et al. [Allard et al., 1990; Pring-Åkerblom et al., 1995]. The 5′-primer was 5′-GCCCCAATGGGCATACATGCACATC-3′ and the 3′-primer was 5′-CAAGCACCCCCCGAATGTCAAAG-3′. With these primers the length of the hexon-specific PCR product was 308 bp, corresponding to amino acids 8–100 of the hexon molecule. An internal standard of 350 bp was constructed by PCR from part of the sequence of the interferon alpha-2 gene with flanking adenovirus sequences complementary to adenovirus-specific primers H1 and H2 [Allard et al., 1990]. This gene fragment was cloned into the EcoRI site of pGEM7Zf(+) plasmid (Promega, Madison, WI). As a positive control we used an Ad2 hexon gene-containing pGEM7Zf(+) plasmid. Several negative control tubes (H_2O instead of the sample) were used in all assays. PCR was performed in a final volume of 50 μl, the reaction mixture containing 50-mM Tris-HCl, pH 8.8, 3.5-mM $MgCl_2$, 15-mM $(NH_4)_2SO_4$, 0.01% gelatin, 0.1% TX-100, 0.2-mM dNTPs, 1 μM of each of the two primers and 1-U Taq-polymerase (Promega). The presence of possible inhibitors was checked using an internal control and different amounts of the samples (from 10 μl to 0.05 μl) in the reactions. After an initial incubation of 5 min at 95°C, 35 amplification cycles were run. Each cycle consisted of a denaturation (30 sec at 94°C), an annealing (1 min at 45°C), and an extension (3 min at 72°C) step. The PCR products were analyzed on 1.8% agarose gels containing ethidium bromide, photographed, and processed for Southern blot hybridization. All the gels were blotted onto nylon filters (Hybond N; Amersham) and hybridized with a hexon probe that was labeled with ^{32}P (Amersham) using a random-primed DNA la-

beling kit (Boehringer Mannheim). The probe was a 600-bp Xho I fragment of the Ad2 hexon gene (nt 1–600).

Time-Resolved Fluoroimmunoassay (TR-FIA)

A TR-FIA method was used for the detection of influenza A, B, parainfluenza 1, 2, 3, respiratory syncytial virus (RSV), and adenovirus-specific antigens [Halonen et al., 1983; Hierholzer et al., 1987, 1989; Waris et al., 1988; Nikkari et al., 1989]. The detection of adenoviruses was based on a pair of hexon-specific monoclonal antibodies provided by the Department of Virology, University of Turku, Finland [Hierholzer et al., 1987]. The capture antibody (Mab 20/11) was obtained in the form of ascitic fluid and the purification of the IgG fraction was performed by FPLC equipment (Fast Performance Liquid Chromatography System, Pharmacia, Sweden) with Q-Sepharose FF (Pharmacia) [Waris et al., 1990]. Coating, postcoating, and washing of the polystyrene microstrips (Labsystems Oy, Helsinki, Finland) and the detection of the adenoviruses with the Eu-labeled antibody (Mab 2/6) were performed [Hierholzer et al., 1987]. The assay buffer consisting of 50-mM Tris-HCl, pH 7.8, 0.9% NaCl, 0.01% NaN_3, 0.5% gelatin, 0.01% Tween 40, and 20-mM N'-diethylene-triaminopentaacetic acid, DTPA, was used for the final dilution of the samples (1:10) in the microtiter plate wells and for the dilution of detector antibody. Enhancement solution was purchased from Wallac Oy (Delfia 1244-105, Wallac Oy, Turku, Finland) and the results were recorded with an Arcus fluorometer, model 1230 (Wallac Oy). The cutoff value for the positivity was the mean of the negative controls plus six standard deviation (SD) units [Waris et al., 1988] and it was usually between 1,000–1,500 cpm.

Enzyme Immunoassay (EIA)

Guinea pigs and rabbits were immunized with hexon antigen, which was purified from adenovirus type 2-infected HeLa cells as described [Petterson et al., 1967]. From the resulting hyperimmune sera immunoglobulins were precipitated twice with ammonium sulfate [Gorevic et al., 1985]. Guinea pig immunoglobulins were used to coat (16 hr at 4°C) microtiter plates (Polysorp, Nunc-Immunoplate, Roskilde, Denmark) at a concentration of 7.5 μg/ml in carbonate buffer, pH 9.6, 200 μl/well. The blocking and washing solutions were as described for TR-FIA, but also 2% normal guinea pig serum was added to the diluent for the rabbit immunoglobulins and the conjugate. NPA specimens diluted to 1:5 and 1:25 were analyzed in duplicates, 150 μl/well for 2 hr at 37°C. Rabbit immunoglobulins were added at a concentration of 10 μg/ml, 150 μl/well for 2 hr at 37°C, followed by alkaline phosphatase-conjugated anti-rabbit IgG (Orion Diagnostica, Espoo, Finland) in incubation conditions described above. P-nitrophenyl phosphate (Sigma) was used as a substrate at 1 mg/ml. Substrate solution (100 μl/well) was incubated for 30 min and the reaction was stopped with 1-N NaOH. Absorbance values were measured with a Multiskan photometer (Labsystems, Helsinki, Finland) at 405 nm. The cutoff values in each assay were determined as the mean OD values of the negative samples (adenovirus culture-negative specimens) plus three SD units.

TABLE I. Positivity Rates of Different Diagnostic Tests for Demonstrating the Presence of Adenovirus in Nasopharyngeal Aspirates From Conscripts With Acute Respiratory Tract Infection and of Serology From Paired Sera

Method	Number of positive specimens/number of samples studied (percentage of positive samples)
Virus culture	159/269 (59.1%)
Rapid virus culture	108/258 (41.9%)
PCR (Ad7)	160/254 (62.9%)
EIA	81/254 (31.9%)
TR-FIA	69/254 (27.1%)
CFT[a]	69/133 (51.9%)

[a]Fourfold or higher increase in adenovirus antibody levels between paired sera.

Serology

The complement fixation test (CFT) for the detection of antiadenovirus antibodies was performed according to the principles of a standard micromethod [Casey et al., 1965]. The antigen was a cell lysate from adenovirus type 1 (Ad1) infected cells and it was produced locally at the Institute.

RESULTS

Viral Infections Among Military Conscripts

Adenoviral infections were by far the most frequently diagnosed infection in our patients. The screening of NPA samples for other respiratory viral antigens by TR-FIA yielded a positive result in 24 other cases: influenza A in 10, influenza B in 8, parainfluenza 3 in 5 cases, and parainfluenza 1 in 1 case. Seven of these cases were double infections together with adenoviruses. The serology of 133 paired sera revealed the following diagnostic antibody rises (= fourfold increase in antibody levels): influenza A in 15 cases, influenza B in 10, and parainfluenza 3 in 1 case. One conscript had diagnostic antibody elevation against three viruses and five had double infections according to the serological test. Reliable comparisons between different diagnostic methods could only be done for adenovirus-specific methods since positivity of adenovirus cultivation or virus-specific antigen or DNA detection was found in 173 specimens out of the total of 269 specimens studied. The positivity rate of the different tests varied from 27% to 63% (Table I), PCR and virus culture showing the highest rates. Serology by CFT detected 69 adenovirus infections (52%) based on diagnostic antibody rises. In 16 cases diagnostic antibody rise was the only sign of adenovirus infection, since tests for the direct detection of adenovirus were negative. All five direct adenovirus-specific tests were carried out on 238 NPA samples and comparison of the different test results are shown in Table II. Apart from those cases where all tests were positive (57 specimens)

TABLE II. Comparison of the Different Diagnostic Tests for Demonstrating the Presence of Adenovirus in Nasopharyngeal Aspirates (NPAs)

Number of NPA specimens	Result of method				
	Virus culture	Rapid virus cultivation	PCR	EIA	TR-FIA
57	+	+	+	+	+
10	+	+	+	+	−
9	+	−	+	+	+
34	+	+	+	−	−
1	+	+	−	−	+
1	+	−	+	+	−
2	+	+	−	−	−
24	+	−	+	−	−
1	+	−	−	+	−
6	+	−	−	−	−
12	−	−	+	−	−
81	−	−	−	−	−
238[a]	146[b]	104[b]	147[b]	78[b]	67[b]

[a]Total number of samples.
[b]Number of positive results.

or all negative (81), the most frequent combinations were positive results in conventional and rapid virus culture and PCR (34 cases), and positive results in virus culture and PCR (24 cases).

Comparison of Conventional and Rapid Adenovirus Culture Methods

Adenovirus cultivation from NPA samples was carried out in A549 tube cell cultures. During the 10- to 14-day culture period, 163 of the 269 specimens induced cytopathic effect. Subculture on coverslips followed by immunofluorescence detection of intracellular hexon antigen to confirm virus culture revealed that nine specimens with a detectable CPE could not be confirmed to contain adenovirus while five CPE negative samples turned out to be adenovirus culture positive. Altogether 159 specimens were considered positive and 110 negative in virus culture. Twenty-five adenovirus culture positive samples originating from patients serving in garrisons located far from each other were serotyped and all of them were found to be of serotype 7. Rapid 2-day culture from the original sample followed by hexon-specific immunostaining revealed adenovirus positivity in 108 (71%) cases (Table III). A cross-comparison of the rapid virus cultivation method with the PCR, EIA, and TR-FIA methods is shown in Table IV.

Detection of Adenoviral DNA by PCR

In order to develop a simple, sensitive, and specific PCR assay for adenovirus DNA, we evaluated both the sample treatment protocols and PCR conditions. The most reliable and sufficiently sensitive sample treatment method was a boiling method in which the sample was boiled for 30 min followed by PCR. Various DNA concentration methods such as ethanol precipitation following proteinase K treatment and phenol extraction or DNA isolation using the QiaAmp Blood Kit (Qiagen, Düsseldorf) gave more varied results (data not shown). Since adenovirus typing revealed serotype 7 in all the specimens studied, we examined NPA specimens with Ad7 hexon-specific primers. A total of 160 samples were positive (63%) out of the 254 samples studied. However, the specificity was 85%, since 15 PCR-positive samples were negative by virus culture (Table III). Out of these 15 PCR-positive specimens 6 corresponding paired serum samples were available and 3 of them showed a diagnostic antibody rise. This suggests that at least these individuals were suffering from an acute adenovirus infection. Cross-comparison of the PCR method with other rapid antigen-specific methods showed the sensitivity of PCR to be superior to the other methods (Table IV).

Adenovirus-Specific EIA and TR-FIA Tests

Adenovirus antigen-specific EIA and one-incubation, monoclonal antibody-based TR-FIA tests were performed on 254 NPA samples. A positive result was obtained in 81 cases by EIA and in 69 cases by TR-FIA. The agreement between the two tests was 95%. All the antigen-positive samples except one in EIA were also found to be virus culture-positive. EIA and TR-FIA antigen detection tests displayed excellent specificity but a relatively low sensitivity of 53% and 46%, respectively (Table III).

Comparison of Direct Virus Detection Methods to Serology

Adenovirus antibody-specific CFT was performed for 133 paired serum samples. Comparison of the number of diagnostic antibody rises with other adenovirus detection methods revealed that CFT showed a positive result in as many cases as were detected by virus culture or PCR. In 41 cases the serological results were in agreement with both virus culture and PCR results from the same patients' NPA specimens, all of them being positive. In the group of conscripts with a significant antibody rise, there were 16 NPA samples, which gave a negative result in both virus culture and in PCR. However, there were many divergent results (Table V), suggesting that both serology and virus detection methods have complementary roles in adenovirus diagnosis.

Detection of Viral Antigen in Relation to Onset of Disease

NPA samples were obtained at different time points in relation to the onset of disease. The percentage of culture and PCR-positive NPA specimens appeared to increase up to the sixth day after the onset of disease and started to decrease thereafter (Fig. 1). At its lowest, virus culture was positive in 28% of the cases studied, at day 1 after onset, but rose rapidly to 55% on the second day after the onset of disease. However, 46.2% of the specimens taken at day 1 after onset were positive by PCR. PCR also showed a higher positivity rate than virus isolation in samples obtained six days or later after the onset of infection.

TABLE III. Comparison of the Diagnostic Efficacies of Adenovirus-Specific Tests With Virus Culture Confirmed With Hexon-Specific Immunostaining in 269 Nasopharyngeal Aspirate, (NPA) Samples

Diagnostic method	Rapid virus culture		PCR		EIA		TR-FIA	
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
Virus culture								
Positive	108	44	145	9	80	71	69	82
Negative	0	106	15	85	1	102	0	103
Total	258		254		254		254	
Sensitivity[a]	71%		94.2%		53%		45.7%	
Specificity[a]	100%		85%		99%		100%	
PPV[a]	100%		90.6%		98.8%		100%	
NPV[a]	70.6%		90.4%		58.9%		55.7%	

[a]Sensitivity, specificity, PPV, and NPV were calculated for each test by comparing the test in question to virus culture. Sensitivity was defined as the number of positives both in the test being evaluated and in virus culture divided by the total number of all positives in virus culture, × 100. Specificity was defined as the number of negatives both in the test being evaluated and in virus culture divided by the number of negatives in virus culture, × 100. The PPV was defined as the number of true positives (positive both in the test being evaluated and in virus culture) divided by the number of positives in the test in question, × 100. The NPV was defined as the number of true negatives (negative both in the test being evaluated and in virus culture) divided by the number of negatives in the test in question, × 100.

TABLE IV. Cross-Comparison of Different Rapid Methods for the Detection of Adenovirus From Nasopharyngeal Aspirates

	PCR		EIA		TR-FIA	
	Positive	Negative	Positive	Negative	Positive	Negative
Rapid virus culture						
Positive	104	2	68	37	59	46
Negative	52	90	12	131	9	134
PCR						
Positive			76	72	68	85
Negative			4	100	0	90
EIA						
Positive					68	13
Negative					1	172

TABLE V. Comparison of CFT Results of Paired Serum Samples With Results From Corresponding Nasopharyngeal Aspirate (NPA) Samples

CFT result	Virus culture		Rapid virus culture		PCR		EIA		TR-FIA	
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
Significant Ab rise[a]	49	20	36	28	45	21	28	37	22	43
No Ab rise	21	43	15	47	22	35	8	54	9	53
Total number of specimens	133		126		123		127		127	

[a]Fourfold or higher rise in adenovirus antibody levels.

DISCUSSION

Etiological diagnosis of an acute respiratory infection is based on the identification of a potentially pathogenic microbe from a respiratory specimen or on demonstrating a diagnostic antibody elevation to a given microbe between paired serum specimens. Rapid etiological diagnosis is of importance in designing the optimal treatment. Conventional and rapid diagnostic methods have been established for respiratory pathogens over the decades. However, in most studies comparison of the diagnostic potential of these methods has been limited. Our clinical specimens, taken from nearly 300 patients during an outbreak of upper respiratory infections, showed that adenovirus was by far the most frequently detected (up to 60% of patients) viral pathogen. This opportunity was therefore used to compare a number of adenovirus-specific diagnostic methods using both NPA and serum specimens.

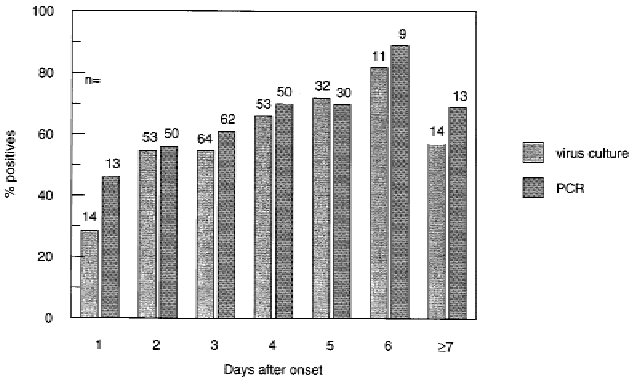

Fig. 1. Percentage of positive adenovirus culture and PCR results in nasopharyngeal aspirate samples in relation to sampling time. The numbers above the bars refer to the total number of specimens in the groups.

Since most adenovirus strains grow in cell culture, our strategy was to culture the virus from NPA samples in order to identify adenovirus-infected individuals. Initially, both Vero and A549 cells were used in tube cell cultures, but since A549 cells seemed to maintain virus growth better than Vero cells, we relied solely on this cell line in our analysis. Although the cytopathic effect caused by adenoviruses in A549 cells was clearly visible, a confirmatory culture step on coverslips was used followed by an immunofluorescence assay with hexon-specific antibodies. Confirmed culture positivity was used as the gold standard for comparisons between different adenovirus-specific tests. Of the 269 NPA samples examined by immunofluorescence-confirmed culture technique, there were nine 9 false positive specimens (positive CPE), but 5 additional samples proved to be adenovirus-positive without apparent CPE positivity. Altogether, adenoviruses was isolated in nearly 60% of cases (Table I). The virus isolation procedure is clinically less applicable than rapid diagnostic methods as it takes up to 2 weeks to perform when compared to the rapid tests, which can be completed in 1 to 2 days. However, the long culture time and a confirmatory step ensured that even small amounts of infectious adenoviruses were detected.

Rapid cultivation directly from NPA samples for 2 days followed by immunofluorescence staining with hexon-specific antibodies also proved to be a very specific and relatively sensitive method for detecting adenoviruses. The specificity was 100%, whereas the sensitivity was approximately 70% when compared to virus isolation. The rapid virus culture method has several advantages: it is fast, small sample series can easily be analyzed, and other respiratory viruses can also be detected using a similar procedure provided that a susceptible cell line and specific antisera are available. It may be less prone to technical problems than PCR or antigen-specific methods and true positive results can be obtained without any further confirmatory testing. EIA and TR-FIA performed almost equally well using this patient material, showing sensitivities of 53% and 46% and specificities of 99% and 100%, respectively. It is concluded that the two antigen detection methods are suitable for the fast detection of adenoviral antigens in clinical specimens. However, direct antigen methods appear to be less sensitive than methods based on virus cultivation [Lehtomäki et al., 1986], whether they are conventional or rapid. This may indicate that rather low amounts of viruses or viral antigens are found in the nasopharyngeal secretions of adenovirus-infected adults. Direct viral antigen-specific methods have, however, proved to be very efficient in establishing fast etiological diagnosis in children [Sarkkinen et al., 1981; Meurman et al., 1983]. This could be due to the more productive nature of adenovirus infection in children.

In recent years, there have been great efforts to develop DNA amplification methods, which are equally sensitive and reliable as the adenovirus cultivation method. Hierholzer et al. [1993] developed a PCR method with a confirmatory TR-FIA assay. Using this method they reported an overall sensitivity of 91% with 40 out of 44 culture-positive samples giving a positive result. In another study by Pring-Åkerblom and Adrian [1994], it was reported that the PCR method was more sensitive than electron microscopy or EIA. However, virus isolation was not carried out in that study. In the PCR assay, a very important step is the pretreatment of the sample. After analyzing the efficacy of several DNA concentration methods we finally used a simple boiling method to release adenoviral DNA without any further purification of nucleic acids. The possible presence of PCR inhibitors in the PCR assay was detected by inclusion of an internal control which was addded to each NPA sample. Only in two cases were inhibitors present in the original samples, and these were excluded from the analysis. A subset of NPA samples were serotyped and since all of them were found to be of serotype 7 (Ad7), we used Ad7 hexon-specific primers in our PCR analysis. There is some sequence variation in the hexon genes of various adenovirus types. These mismatches may affect the PCR results impairing its sensitivity as has been reported by Morris et al. [1996]. The nucleotide sequences of our Ad 7-specific primers originated from the same positions of the Ad7 hexon gene as the Ad2 primers used by Allard et al. [1990]. In the present study we did not systematically test the efficacy of the primers for the detection of other adenovirus serotypes. The PCR method we used with a simple template pretreatment and a confirmatory hybridization step resulted in 160 positives of the 254 specimens studied with a sensitivity of 94% and a specificity of 85%. There were 15 additional PCR-positive specimens that could not be detected by virus cultivation. These specimens may be true positives that remained below the detection level of the virus culture methods or may represent some noncultivable (or noninfectious) adenovirus types. It is unlikely that they represented false positive PCR samples due to contamination, although we cannot exclude this possibility. In all the series the negative control specimens had remained negative. We also strove to sensitize our PCR assay by using a nested PCR reaction. This was time-consuming and expensive, and it was difficult to avoid false positive results in all assays. On the whole, we feel that the second round of PCR does not significantly improve the applicability of PCR for adenovirus detection. In terms of adenovirus PCR, the key issue is thus the selection of broad-spectrum primer pairs that can detect all the serotypes relevant for diagnostics in humans.

Using a group-specific adenovirus antigen in a complement fixation test, antibodies were measured against adenoviruses in 133 paired serum samples. Of these paired sera there were 69 positive cases, with a significant (fourfold or higher) antibody rise. In 70% of virus isolation-positive cases there was also a diagnostic antibody rise. If individuals with a stable high antibody level were also taken into account, the figure was as high as 76%. This result agrees well with pre-

vious studies [Julkunen et al., 1986; Horwitz, 1996]. It is possible that the presence of adenovirus in the upper respiratory tract does not always elicit antibody responses or alternatively they are not measurable by CFT. On the other hand, there were an additional 20 individuals with a diagnostic antibody rise between paired serum specimens but whose NPA specimens remained negative by virus culture. This discrepancy may be due to an inadequate sample timing, an inadequately collected NPA sample, or a lack of infectivity of the virus in the sample. Alternatively, these patients suffered from an adenovirus infection but failed to secrete the virus at levels detectable by virus culture or any of the viral antigen or DNA detection methods.

In the present study PCR was shown to be a very sensitive method for the detection of adenovirus-specific DNA in nasopharyngeal specimens in adults. In terms of safety, the confirmatory step carried out by hybridization using a radioactive probe should be replaced by a nonradioactive assay. Rapid virus culture also proved to be a very efficient rapid diagnostic method due to its simplicity and a very good specificity. Conventional serological diagnosis also has an important diagnostic role, e.g., for epidemiological and etiological studies, when rapid diagnosis is not required or if NPA specimens are not available. It appears that, in order to detect all possible adenovirus infections, methods based on several different principles should be applied.

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